Exhibit 10

Mechanisms of action of *N*-nitroso compounds

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Summary

There is ample evidence from studies in experimental animals that *N*-nitroso compounds are carcinogenic because in the body they form potent electrophilic alkylating agents. These reactive intermediates are formed by spontaneous decomposition in the case of nitrosoureas and related compounds, or by metabolic activation in the case of *N*-nitrosamines. The electrophiles subsequently react with DNA of target tissues to form altered bases which leads to the initiation of carcinogenesis. There is now convincing evidence that the biological activity of *N*-nitroso compounds in humans does not differ substantially from that in experimental animals. We can therefore predict with a high degree of confidence that *N*-nitroso compounds including nitrosamines are carcinogenic in man.

I Bioactivation of *N*-nitroso compounds

N-nitrosoureas and related compounds (nitrosamides, nitrosoguanidines, nitrosourethanes, nitrosocyanamides) are chemically reactive, and decompose at physiological pH to form electrophilic alkylating agents as shown in Fig. 1. *N*-nitrosamines, on the other hand, are stable at neutral pH, and require metabolic transformation in vivo in order to exert their carcinogenic effects. This difference explains why nitrosoureas tend to produce tumours at

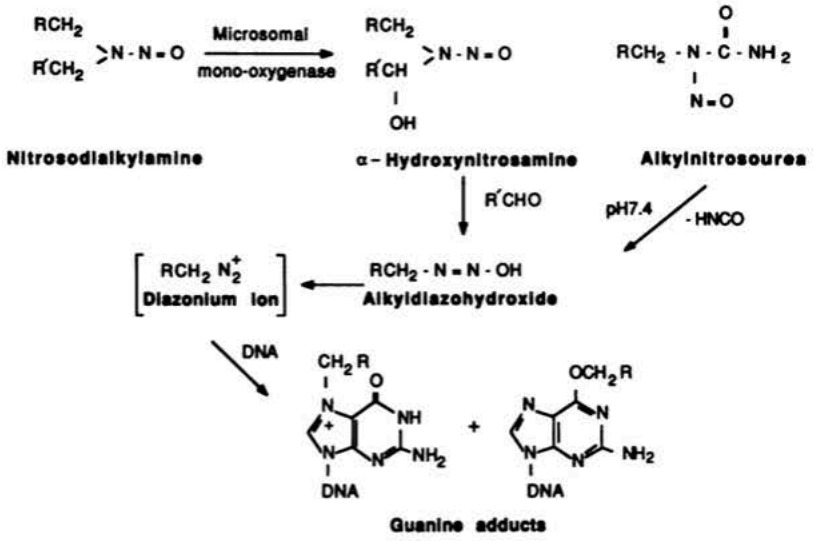

Fig. 1 Bioactivation of *N*-nitroso compounds

or near the site of application whereas nitrosamines produce tumours in tissues remote from the site of administration.

Early experiments (reviewed by Magee and Barnes, 1967, and Druckrey *et al*, 1967) on the pharmacokinetics and in vivo and in vitro metabolism of nitrosamines suggested that these compounds are metabolically activated according to the reaction sequence shown in Fig. 1. Cytochrome P450-dependent hydroxylation at the carbon atom adjacent to the *N*-nitroso group is the critical initial step in the biotransformation. Spontaneous cleavage of the carbon-nitrogen bond in the α-hydroxynitrosamine leads to the production of an aldehyde and the alkyldiazohydroxide. The diazohydroxide finally produces the potent electrophilic alkyl diazonium ion, which may react either with water to form an alcohol or at a nucleophilic site on a biomolecule such as DNA.

Recent experimentation (reviewed by Archer and Labuc, 1985) has substantiated this activation pathway for symmetric and asymmetric nitrosodialkylamines and for cyclic nitrosamines. Metabolic products such as aldehydes, alcohols and molecular nitrogen have been unequivocally identified and quantified for numerous nitrosamines in studies in the whole animal and using cell-free preparations from various organs. Studies of the chemical and biological properties of stable acetoxy derivatives of α-hydroxynitrosamines have provided further support for the activation pathway. Nitrosomethyl(acetoxymethyl)amine was first prepared by Roller *et al* (1975), who showed that solvolysis of this compound led to the formation of an equimolar mixture of acetic acid, formaldehyde and methanol, as predicted from the activation of nitrosodimethylamine (Fig. 1). Esterases that are ubiquitously

distributed in tissues are responsible for the bioactivation of α-acetoxynitrosamine in vivo. The reactive alkylating agent produced from nitrosomethyl (acetoxymethyl)amine by the action of esterases was shown to yield DNA methylation products identical to those produced by nitrosodimethylamine in rat liver (Kleihues *et al*, 1979). As expected, nitrosomethyl(acetoxymethyl) amine produces tumours at or near the site of application or in those tissues first exposed to the compound by systemic circulation (eg Habs *et al*, 1978; Berman *et al*, 1979), and α-acetoxynitrosamines are potent bacterial mutagens that do not require microsomal activation (eg Camus *et al*, 1978; Mochizuki *et al*, 1979). However, the isolation and characterization of free α-hydroxynitrosamines is perhaps the best evidence for the activation mechanism (Mochizuki *et al*, 1980a).

In addition to α-oxidation, two other metabolic reactions, β-oxidation and ω-oxidation, are involved in the activation of a number of nitrosamines. In 1971, Krüger discovered that in addition to the direct transfer of an intact propyl group to DNA, nitrosodipropylamine also acts as a methylating agent. Indeed, 7–methylguanine is the major alkylation product in hepatic DNA following nitrosodipropylamine administration to rats. The mechanism of this reaction involves two consecutive β-oxidation reactions to yield nitroso–2–oxopropylpropylamine (Fig. 2) (Leung and Archer, 1984). Metabolic α-hydroxylation of this nitrosamine on the propyl side chain with loss of propionaldehyde then forms 2–oxopropyldiazotate. This intermediate undergoes internal cyclization to yield an oxadiazoline which finally breaks down to yield acetic acid and the methylating agent diazomethane. Reactions such as these may also explain the ability of nitrosobis(2–hydroxypropyl)amine, nitroso(2–hydroxypropyl)(2–oxopropyl)amine and nitrosobis(2–oxopropyl) amine to methylate DNA (Lawson *et al*, 1981; Lijinsky, 1985). These nitrosamines are important because they induce pancreatic tumours, particularly in the Syrian hamster, that are very similar to the pancreatic tumours that are common in humans (reviewed by Pour and Wilson, 1980).

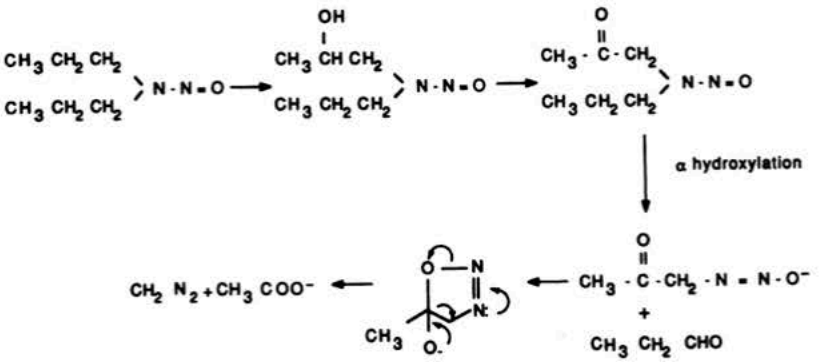

Fig. 2 Mechanism for formation of a methylating agent from nitroso dipropylamine

Nitrosodibutylamine and nitrosobutyl(4–hydroxybutyl)amine, which induce bladder tumours in the rat, have been shown to undergo ω, ω–1 and ω–2, oxidations (reviewed by Okada, 1984). Nitrosobutyl–3–carboxypropylamine is a major urinary metabolite, and probably the proximate carcinogenic form of these nitrosamines. Mochizuki *et al* (1980b) have suggested that the epithelial cells of the bladder activate the nitrosobutyl–3–carboxypropylamine by α-hydroxylation on the 3–carboxypropyl chain, and the resulting α-hydroxynitrosamine is stabilized as the γ-lactone.

Metabolic ω-oxidation reactions followed by β-oxidation have been shown to lead to loss of a two carbon fragment from one side chain of nitrosodibutylamine in a manner analogous to the Knoop mechanism of fatty acid metabolism (Okada, 1984). On the basis of this mechanism, Okada *et al* (1976) proposed that nitrosomethylalkylamines with an even number of carbon atoms would give rise to nitrosomethyl–3–carboxypropylamine, and would be bladder carcinogens, whereas those with an odd number of carbon atoms would not produce the 3–carboxypropyl derivative, and hence would not induce bladder tumours. This prediction has been supported by carcinogenesis studies with a series of nitrosomethylalkylamines (Lijinsky *et al*, 1981).

In addition to metabolic activation reactions, nitrosamines may also be deactivated by metabolism. The best characterized detoxification pathway is denitrosation (Keefer *et al*, 1987 and references therein), although there is some evidence for reduction to corresponding asymmetrical hydrazines (Grilli and Prodi, 1975).

II Fate of reactive intermediates

The electrophilic intermediates produced by chemical decomposition of nitrosoureas and related compounds, or by metabolic activation of nitrosamines, react rapidly with cellular nucleophiles. Attention has focused on reactions with DNA because this is generally considered to be the critical cellular target for carcinogens during tumour initiation.

Although 7–alkylguanine (Fig. 1) (66.8%) is the most abundant modified base in DNA produced by nitrosodialkylamines, a variety of other products have been identified. These include alkylphosphate triesters (12%); 1–, 3– and 7–alkyladenine (0.9%, 2.3%, 0.7%); 3– and O^6–alkylguanine (Fig. 1) (0.9%, 6.1%); 3–alkylcytosine (0.6%); O^4–alkylthymine (trace); and unidentified products (10%) (the numbers in parentheses are the relative proportions of the products as a percentage of the total products for methylation of DNA in rat liver by nitrosodimethylamine, reported by O'Connor *et al*, 1979). Not all of these DNA lesions, however, have the same biological importance. Methylation of the 7–position of guanine shows no correlation with carcinogenic activity, but several striking correlations have been obtained between tissue susceptibility to tumour induction and the initial extent of formation and subsequent persistence of O^6–methylguanine residues (reviewed by Pegg, 1983). There is also some evidence that O^4–ethylthymine may be an important lesion for animals continuously exposed to nitrosodiethylamine (Dyroff *et al*, 1986).

Since tumour initiation is probably caused by replication of an unrepaired DNA lesion, DNA repair enzymes undoubtedly have an important role in carcinogenesis. Guanine alkylated at the O^6 position is repaired in an unusual process in which the alkyl group is actually transferred from the base to a cysteine residue in the repair enzyme (Pegg, 1983). For each base repaired, a molecule of O^6–alkylguanine-DNA alkyltransferase is irreversibly inactivated by the process. Human cells in culture and human liver generally have a higher capacity to repair O^6–methylguanine than rodent cells or rodent liver (Montesano and Wild, 1988 and references therein), a factor that may be important in extrapolating from carcinogenesis studies in rodents to humans. Subpopulations of individuals deficient in the ability to repair such DNA lesions, however, may be particularly prone to developing cancer.

The importance of O^6–alkylguanine in carcinogenesis has been highlighted by recent experiments on the molecular biology of cancer induction by *N*-nitroso compounds. A high percentage of mammary tumours induced in rats by methylnitrosourea contained Ha-*ras* oncogenes which were activated by G to A transitions in the 12th codon (Zarbl *et al*, 1985). This transition has been shown to be caused by O^6–methylguanine mispairing with thymine during DNA replication (Loechler *et al*, 1984).

III Evidence for the carcinogenic activity of *N*-nitroso compounds in humans

Although there is no unequivocal evidence for the carcinogenic activity of *N*-nitroso compounds in humans, a number of different lines of evidence suggest that humans are not resistant to the effects of these compounds.

1 Nitrosourea chemotherapy

Three chloroethylnitrosourea drugs, carmustine/BCNU (1,3–bis(2–chloroethyl)–1–nitrosourea), lomustine/CCNU (1–(2–chloroethyl)–3–cyclohexyl–1–nitrosourea) and semustine/methyl-CCNU (1–(2–chloroethyl)–3–(4–methylcyclohexyl)–1–nitrososurea), have been used for more than a decade to treat patients with malignant melanoma and advanced cancers of the brain, lung and digestive tract. These are antitumour drugs because they are alkylating agents, but this property makes the nitrosoureas carcinogenic. Several recent studies of patients who have undergone cancer chemotherapy have shown that nitrosourea treatment is associated with the subsequent development of acute non-lymphocytic leukaemia and preleukaemia (Boice *et al*, 1983, 1986 and references therein).

2 Nitrosamine poisonings

In 1937, Freund investigated two cases of human poisoning by nitrosodimethylamine caused by industrial exposure. He reported finding toxic parenchymatous hepatitis with ascites. Similar findings in occupationally

exposed workers were reported by Barnes and Magee (1954). More recently, in a case of deliberate poisoning by nitrosodimethylamine in Germany, the victim died of liver decomposition and cirrhosis (Fussganger and Ditschuneit, 1980). The hepatotoxic effects seen in these cases are similar, if not identical, to the acute toxic effects of nitrosodimethylamine seen in rodents (Magee and Barnes, 1967). In a case of poisoning by the same nitrosamine in the United States, Herron and Shank (1980) were able to show the presence of 7–methylguanine and 0^6–methylguanine in the DNA of a sample of the victim's liver. It is clear, therefore, that humans, like rodents, activate nitrosodimethylamine to a methylating agent.

3 Comparative in vitro metabolism

Several studies have compared the metabolism of nitrosamines or their binding to DNA in human tissue samples and rodents in which the compounds are known to be carcinogenic. Montesano and Magee (1974) compared the metabolism of *N*-nitrosodimethylamine in liver slices from various species including humans. Measuring CO_2 evolution and 7–methylguanine formation in DNA, they showed that human liver is similar to rat liver in its ability to metabolize nitrosodimethylamine (Table). Liver slices from monkey and trout, which are relatively resistant to the carcinogenic activity of nitrosodimethylamine (Ashley and Halver, 1968; Adamson and Sieber, 1983), possess significantly lower metabolic activities. Microsomes from human liver samples have been shown to metabolize nitrosamines (Lin and Fong, 1980; Yoo *et al*, 1988). Yoo *et al* (1988) showed that human liver microsomes are as efficient as rat liver microsomes in the metabolism of nitrosodimethylamine. They also showed that human liver microsomes catalyse the dealkylation and denitrosation of several other nitrosamines. Furthermore, human liver and lung fractions have been shown to convert nitrosamines into metabolites that are mutagenic for bacteria (Czygan *et al*, 1973; Bartsch *et al*, 1976; Sabadie *et al*, 1980).

4 Studies with cultured human tissues and cells

Explant cultures from a variety of different human tissues including bronchus, oesophagus, urinary bladder, colon and pancreatic duct have been used,

Comparative metabolism of *N*-nitrosodimethylamine in liver tissue slices from various species, including humans

Species	*Relative activity*[a]
Hamster (Syrian golden)	100
Rat	65
Human	45
Monkey	6.1
Trout	0.1

[a] Expressed as percentage of the formation of 7-methylguanine and [^{14}C] carbon dioxide observed in hamster. Data from Montesano and Magee (1974) adapted by Bartsch and Montesano (1984)

particularly by Harris, Autrup and coworkers (Harris *et al*, 1982 and references therein) to study nitrosamine metabolism and DNA binding. Nitrosodimethylamine and nitrosodiethylamine were metabolized in all tissues examined, but some tissues were unable to metabolize cyclic and asymmetric dialkyl nitrosamines. There were large quantitative differences in metabolic rates (up to 150–fold) among individuals. In a few instances, DNA methylation by nitrosodimethylamine in human explant cultures has been observed. Furthermore, in vitro transformation of cultures of human pancreatic epithelial cells by nitrosodimethylamine and methylnitrosourea has been reported (Parsa *et al*, 1981).

In conclusion, the results of these various studies suggest that the biological activity of *N*nitroso compounds is similar in humans and experimental animals. It seems unlikely, therefore, that humans will be resistant to the carcinogenic action of these compounds.

References

Adamson RH and Sieber SM (1983) Chemical carcinogenesis studies in nonhuman primates. In: R Lagenbach, S Nesnow and JM Rice (eds), *Organ and Species Specificity in Chemical Carcinogenesis*, pp 129–156. Plenum Press, New York

Archer MC and Labuc GE (1985) Nitrosamines. In: MW Anders (ed), *Bioactivation of Foreign Compounds*, pp 403–431. Academic Press, Orlando FL

Ashley LM and Halver JE (1968) Dimethylnitrosamine-induced hepatic cell carcinoma in rainbow trout. *Journal of the National Cancer Institute* **41** 531–552

Barnes JM and Magee PN (1954) Some toxic properties of dimethylnitrosamine. *British Journal of Industrial Medicine* **11** 167–174

Bartsch H and Montesano R (1984) Relevance of nitrosamines to human cancer. *Carcinogenesis* **5** 1381–1393

Bartsch H, Camus A and Malaveille C (1976) Comparative mutagenicity of N-nitrosamines in a semi-solid and in a liquid incubation system in the presence of rat or human tissue fractions. *Mutation Research* **37** 149–162

Berman JJ, Rice JM, Wenk ML and Roller PP (1979) Dependence of tumor spectrum on route of administration in Sprague-Dawley rats as a result of single or multiple injections of methyl(acetoxymethyl)nitrosamine. *Journal of the National Cancer Institute* **63** 93–100

Boice JD, Green MH, Killen JY, Ellenberg SS, Keehn RJ, McFadden E, Chen TT and Fraumeni JF (1983) Leukemia and preleukemia after adjuvant treatment of gastrointestinal cancer with semustine (methyl-CCNU). *New England Journal of Medicine* **309** 1079–1084

Boice JD, Green MH, Killen JY, Ellenberg SS, Fraumeni JF, Keehn RJ, McFadden E, Chen TT and Stablein D (1986) Leukemia after adjuvant chemotherapy with semustine (methyl-CCNU)—evidence of a dose-response effect. *New England Journal of Medicine* **314** 119–120

Camus AM, Wiessler M, Malaveille C and Bartsch H (1978) High mutagenicity of N-(α-acyloxy)alkyl-N-alkylnitrosamines in S. typhimurium: model compounds for metabolically activated N, N-dialkylnitrosamines. *Mutation Research* **49** 187–194

Czygan P, Greim H, Garro AJ, Hutterer F, Rudick J, Schaffner F and Popper H (1973) Cytochrome P–450 content and the ability of liver microsomes from patients

undergoing abdominal surgery to alter the mutagenicity of a primary and secondary carcinogen. *Journal of the National Cancer Institute* **51** 1761–1764

Druckrey H, Preussmann R, Ivankovic S and Schmähl D (1967) Organotrope carcinogene Wirkungen bei 65 verschiedenen N-Nitroso-verbindungen an BD-Ratten. *Zeitschrift für Krebsforschung* **69** 103–201

Dyroff MC, Richardson FC, Popp JA, Dedell MA and Swenberg JA (1986) Correlation of 0^4–ethyldeoxythymidine accumulation, hepatic initiation and hepatocellular carcinoma induction in rats continuously administered diethylnitrosamine. *Carcinogenesis* **7** 241–246

Freund HA (1937) Clinical manifestations and studies in parenchymatous hepatitis *Annals of Internal Medicine* **10** 1114–1115.

Fussganger RD and Ditschuneit H (1980) Lethal exitus of a patient with N-nitrosodimethylamine poisoning, 2–3 years following the first ingestion and signs of intoxication. *Oncology* **37** 273–277

Grilli S and Prodi G (1975) Identification of dimethylnitrosamine metabolites in vitro. *Gann* **66** 473–480

Habs M, Schmähl D and Wiessler M (1978) Carcinogenicity of acetoxymethylmethylnitrosamine after subcutaneous, intravenous and intrarectal application in rats. *Zeitschrift für Krebsforschung* **91** 217–221.

Harris CC, Grafstrom RC, Lechner JF and Autrup H (1982) Metabolism of N-nitrosamines and repair of DNA damage in cultured human tissues and cells. In: PN Magee (ed), *Nitrosamines and Human Cancer*, Banbury Report 12. Cold Spring Harbor Laboratory, New York pp 121–139.

Herron DC and Shank RC (1980) Methylated purines in human liver DNA after probable dimethylnitrosamine poisoning. *Cancer Research* **40** 3116–3117.

Keefer LK, Anjo T, Wade D, Wang T and Yang CS (1987) Concurrent generation of methylamine and nitrite during denitrosation of N-nitrosodimethylamine by rat liver microsomes. *Cancer Research* **47** 447–452

Kleihues P, Doerjer G, Keefer LK, Rice JM, Roller PP and Hodgson RM (1979) Correlation of DNA methylation by methyl(acetoxymethyl)nitrosamine with organ-specific carcinogenicity in rats. *Cancer Research* **39** 5136–5140

Krüger FW (1971) Metabolismus von Nitrosaminen in vivo. I. Uber die β–Oxidation Aliphatischer Di-n-alkylnitrosamine: Die Bildung von 7–Methylguanine neben 7–Propylbzw. 7–Butylguanin nach Applikation von Di-n-propyl-oder Di-n-butylnitrosamin. *Zeitschrift für Krebsforschung* **76** 145–154

Lawson TA, Gingell R, Nagel D, Hines LA and Ross A (1981) Methylation of hamster DNA by the carcinogen N-nitroso-bis(2–oxopropyl)amine. *Cancer Letters* **11** 251–255

Leung KH and Archer MC (1984) Studies on the metabolic activation of β–keto nitrosamines: mechanisms of DNA methylation by N–(2–oxopropyl)–N-nitrosourea and N-nitroso-N-acetoxymethyl–N–2–oxopropylamine. *Chemico-Biological Interactions* **48** 169–179

Lijinsky W (1985) The metabolism and cellular interactions of some aliphatic nitrogenous carcinogens. *Cancer Letters* **26** 33–42

Lijinsky W, Saavedra JE and Reuber MD (1981) Induction of carcinogenesis in Fischer rats by methylalkylnitrosamines. *Cancer Research* **41** 1288–1292

Lin JH and Fong LYY (1980) Effects of oxygen depletion on in vitro metabolism of dimethylnitrosamine in microsomes from rat liver and human tissues. *Journal of the National Cancer Institute* **65** 877–883

Loechler EL, Green CC and Essigman JM (1984) In vitro mutagenesis by 0^6–

methylguanine built into a unique site in a viral genome. *Proceedings of the National Academy of Sciences of the USA* **81** 6271–6275

Magee PN and Barnes JM (1967) Carcinogenic nitroso compounds. *Advances in Cancer Research* **10** 163–246

Mochizuki M, Suzuki E, Anjo T, Wakabayashi Y and Okada M (1979) Mutagenic and DNA-damaging effects of N-alkyl-N-(α-acetoxyalkyl)nitrosamines, models for metabolically activated N, N-dialkylnitrosamines. *Gann* **70** 663–670

Mochizuki M, Anjo T and Okada M (1980a) Isolation and characterization of N-alkyl-N-(hydroxymethyl)nitrosamines from N-alkyl-N-(hydroperoxymethyl)nitrosamines by deoxygenation. *Tetrahedron Letters* **21** 3693–3696

Mochizuki M, Irving CC, Anjo T, Wakabayashi Y, Suzuki E and Okada M (1980b) Synthesis and mutagenicity of 4–(N–butylnitrosamino)–4–hydroxy-butyric acid lactone, a possible activated metabolite of the proximate bladder carcinogen N-butyl-N-(3–carboxypropyl)nitrosamine. *Cancer Research* **40** 162–165

Montesano R and Magee PN (1974) Comparative metabolism in vitro of nitrosamines in various animal species including man. In: R Montesano and L Tomatis (eds), *Chemical Carcinogenesis Essays*, pp 39–56. International Agency for Research on Cancer, Scientific Publication No 10, Lyon

Montesano R and Wild CP (1988) Repair of alkylated DNA in rodent and human tissues. In: OH Iversen (ed), *Theories of Carcinogenesis*, pp 99–117. Hemisphere, Washington DC

O'Connor PJ, Saffhill R and Margison GP (1979) N-Nitroso compounds: biochemical mechanisms of action. In: P Emmelot and E Kriek (eds), *Environmental Carcinogenesis*, pp 73–96. Elsevier/North Holland, Amsterdam

Okada M (1984) Comparative metabolism of N-nitrosamines in relation to their organ and species specificity. In: IK O'Neill, RC Von Borstel, CT Miller, J Long and H Bartsch (eds), *N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer*, pp 401–409. International Agency for Research on Cancer, Scientific Publication No 57, Lyon

Okada M, Suzuki E and Mochizuki M (1976) Possible important role of urinary N-methyl-N-(3–carboxypropyl)nitrosamine in the induction of bladder tumors in rats by N-methyl-N-dodecyl-nitrosamine. *Gann* **67** 771–772

Parsa I, Marsh WH and Sutton AC (1981) An in vitro model of human pancreas carcinogenesis: effects of nitroso compounds. *Cancer* **47** 1543–1551

Pegg AE (1983) Alkylation and subsequent repair of DNA after exposure to dimethylnitrosamine and related compounds. In: E Hodgson, JR Bend and RM Philpot (eds), *Reviews in Biochemical Toxicology*, Vol 5, pp 83–133. Elsevier, New York

Pour PM and Wilson RB (1980) Experimental tumors of the pancreas. In: AR Moossa (ed), *Tumors of the Pancreas*, pp 37–158. Williams and Wilkins, Baltimore

Roller PP, Shimp DR and Keefer LK (1975) Synthesis and solvolysis of methyl(acetoxymethyl)nitrosamine. Solution chemistry of the presumed carcinogenic metabolite of dimethylnitrosamine. *Tetrahedron Letters* **25** 2065–2068

Sabadie N, Malaveille C, Camus A and Bartsch H (1980) Comparison of the hydroxylation of benzo(a)pyrene with the metabolism of vinyl chloride, N-nitrosomorpholine, and N-nitroso-N′-methylpiperazine to mutagens by human and rat liver microsomal fractions. *Cancer Research* **40** 119–126

Yoo J-SH, Guengerich FP and Yang CS (1988) Metabolism of N-nitrosodialkylamines by human liver microsomes. *Cancer Research* **88** 1499–1504

Zarbl H, Sukumar S, Arthur AV, Martin-Zanca D and Barbacid M (1985) Direct

mutagenesis of Ha-ras-1 oncogenes by N-nitroso-N-methylurea during initiation of mammary carcinogenesis in rats. *Nature* **315** 382–385

(The author is responsible for the accuracy of the references.)

Environmental exposure to preformed nitroso compounds

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Summary

In the human environment, nitrosatable amine precursors to *N*-nitroso compounds and nitrosating species such as nitrite and oxides of nitrogen are abundant. As a result, the formation of *N*-nitroso compounds and human exposure to these compounds show a rather complex pattern. The largest known human exposures to exogenous *N*-nitrosamines occur in the work place. This is particularly evident in the rubber and tyre manufacturing industry and in metal cutting and grinding shops. Nearly all industries which are concerned with the production and/or use of amines have a related nitrosamine problem. Outside the industrial environment, commodities such as cosmetics, pharmaceuticals, rubber and household products, which are

